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FURTHER STUDIES OF ANTISTREPTOCOCCUS SERUM.*

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In June, 1909, in the Section of Pharmacology and Therapeutics of the American Medical Association,[1] together with Dr. L. Hektoen, we made a brief preliminary report on a study of various commercial antiserums and vaccines. An immediate resumption of the investigation was prevented by other work, but during the past few months a further study of antistreptococcus serums was undertaken, and the results obtained and now reported are in some respects at variance with those secured in our former work. In the earlier experiments we failed to demonstrate that animals into which antistreptococcus serums were injected possessed any increased resistance to subsequent injections of streptococci. Our efforts to demonstrate streptococco-opsonins in the serums usually met with negative results. At times we were inclined to think that opsonic determinations made by Wright's method pointed toward the presence of specific opsonins in the serums when mixed with fresh serum. However, the opsonic indexes which were obtained were so little above the normal and appeared so inconstant that they were considered as coming within the limits of error in the method employed. The same was true as regards determinations of opsonins in the blood of animals after injections of antistreptococcus serums.

METHODS EMPLOYED.

In the studies now reported guinea-pigs have been employed exclusively for the tests because of the relative ease with which they are secured in sufficient number and of a uniform size. The streptococcus used is designated "Streptococcus S"; it had been rendered quite virulent for guinea-pigs by multiple passages. The minimum lethal dose of a 24-hour broth culture of this organism for medium sized guinea-pigs is from 0.02 to 0.03 c.c. The culture is kept

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* Received for publication June 5, 1911.

[1] "Preliminary Report of Investigations of Serums and Vaccines for Streptococcus, Staphylococcus, and Pneumococcus Infections," *Jour. Am. Med. Assn.*, 1910, 54, p. 257.

on blood agar and its virulence remained unaltered during the time covered by our experiments. At intervals of about a month the stock culture is transferred to fresh slants of blood agar and, after being allowed to grow for 24 hours at 35° C., kept in the ice-box. The fresh cultures used in the experiments were secured by inoculations from such stock cultures. The broth employed was made sugar-free by Theobald Smith's method, and 0.2 per cent of dextrose was added to it before sterilization.

The opsonic determinations have been made with a modified Wright's technic which after long use yields very constant results with slight degree of error. Estimation of the opsonin by dilution to the point of opsonic extinction was also employed. The virulent "Streptococcus S" was used in these tests, and undergoes very little phagocytosis when treated with normal serum. Human leukocytes were used in all cases unless otherwise stated.

We take this opportunity of expressing our obligation to Dr. E. M. Houghton, director of the Biological and Research Departments of Parke, Davis & Co., for supplying us with fresh polyvalent antistreptococcus serums free from preservatives. Serums from three individual horses were sent, packed in ice, each month as soon as possible after the bleedings. These serums have been largely used in our study and are designated by the numbers 891, 893, and 1,130. Unless otherwise stated they contain no preservative.

EXAMINATION OF THE SERUMS OF INDIVIDUAL HORSES.

The serums of the three horses obtained at four successive monthly bleedings were examined for opsonin five to 10 days after being drawn. The amount of opsonin at this primary examination as determined by the usual method and by dilution to the point of opsonic extinction is shown in Table 1. The results secured by the two methods of estimation correspond quite closely. It will be noted that the amount varies considerably among the horses and also in the same horses at different bleedings. Some of the serums had been kept longer than others after being drawn because of delay in transportation, so that the variation may be greater than would be the case if the examinations had been made immediately after the blood was drawn.

TABLE 1.*

SHOWING THE OPSONIC POWER OF THE SERUMS OF THREE HORSES SOON AFTER BLEEDING IN FOUR SUCCESSIVE MONTHS.

Date of Bleeding	No. of Horse	No. of Leukocytes out of 50 Taking Part	Average No. of Cocci per Phagocyte	Point of Opsonic Extinction
October, 1910........	891	29	4.3	1:768
	893	12	1.7	1:6
	1,130	23	4.2	1:192
November, 1910......	891	22	3.2	1:768
	893	11	0.8	1:24
	1,130	25	3.9	1:768
December, 1910......	891	15	1.4	1:192
	893	9	0.7	1:24
	1,130	1	0.4	1:12
Jaunary, 1911........	891	24	4.0	1:384
	893	26	4.1	1:768
	1,130	25	4.4	1:384

* The amount of phagocytosis with normal horse serum was almost nil, scarcely more than with salt solution.

October serums were examined 6 days after bleeding.
November " " " 8 " " "
December " " " 10 " " "
January " " " 6 " " "

PERSISTENCE OF OPSONIC POWER.

At intervals of about two weeks the examinations were repeated. It was soon evident that the opsonic power progressively and rapidly disappeared from serums kept in the ice-box, and at the end of a month all the serums had lost their property of opsonizing virulent streptococci. The rapidity with which this occurred in

TABLE 2.*

SHOWING THE PERIODS DURING WHICH THE SERUMS FROM THREE HORSES IN FOUR SUCCESSIVE MONTHS RETAINED THEIR OPSONIC ACTIVITY AND THE PERIOD DURING WHICH THE SAME SERUMS COULD BE REACTIVATED BY FRESH SERUM.

Date of Bleeding	No. of Horse	Point of Opsonic Extinction When First Examined	Latest Day after the Bleeding When the Opsonic Power Was Demonstrated in Serum	Latest Time after Bleeding When Reactivation Was Accomplished by Adding Fresh Normal Human Serum
October, 1910....	891	1:768	20 days	29 days
	893	1:6	15 "	21 "
	1,130	1:192	30 "	4 months
November, 1910..	891	1:768	13 days	28 days
	893	1:24	9 "	0 "
	1,130	1:768	21 "	4 months+
December, 1910..	891	1:192	27 "	2 months
	893	1:24	20 "	58 days
	1,130	1:12	27 "	2 months
January, 1911....	891	1:384	13 days	45 days
	893	1:768	13 "	24 "
	1,130	1:384	24 "	2 months+

* October serums were first examined 6 days after bleeding.
November " " " " 8 " " "
December " " " " 10 " " "
January " " " " 6 " " "

the various serums is shown in Table 2. It is fair to infer that considerable loss in opsonic power occurred during the six to 10 days immediately following the drawing of the bloods, and that the serums which were examined longest after the bleeding had lost relatively most. This would account for the smaller opsonic content of the serums which were primarily examined from two to four days later than the other two sets.

THE REACTIVATION OF SERUM.

When the serums had lost their opsonic power as tested in the manner indicated, efforts were made to restore their opsonizing power by the addition of fresh normal serum, human and guinea-pig. The serum of the guinea-pig was chosen because it was the animal used in testing the protective power of the serums, and human serum because antistreptococcus serums are employed therapeutically in man. In these experiments nine parts of fresh normal serum were added to one part of the serum to be tested. It was found that soon after the opsonic power disappeared from the serums, an opsonic activity almost equal to the original could be secured by the addition of fresh normal serum. For some reason the serum from horse 1,130 retained its property of being reactivated by normal serum longer than did the serums of the other horses (see Table 2).

The property of reactivating antistreptococcus serum is possessed in a higher degree by fresh normal human serum than by fresh guinea-pig serum. In the subjoined experiment reactivation of the immune serum was accomplished by human serum when the immune serum in the mixture was to the normal serum as 1:160, while activation by guinea-pig serum occurred only when the proportion was as 1:10 (see Table 3).

NATURE OF ANTIBODIES.

Heating antistreptococcus serums to 60° C. for one-half hour does not affect their subsequent reactivation by fresh normal serums, but fresh human or guinea-pig serums heated to 60° for one-half hour have lost their power of reactivating old antistreptococcus serums. This appears to point to the complement of the fresh serum as the essential factor in the reactivation. Aronson[1] has pointed out that the antibodies are of the class of Ehrlich's amboceptors, withstanding 62°–63° for one hour and not being injured by 0.4 per cent trikresol.

TABLE 3.

Antistreptococcus Serum	Salt Solution	Fresh Normal Human Serum	Fresh Normal Guinea-Pig Serum	Average No. of Cocci per Phagocyte
0.0	0.1	0.9	...	0.64
0.1	0.0	0.9	...	2.1
0.05	0.05	0.9	...	1.9
0.025	0.075	0.9	...	2.6
0.0125	0.0875	0.9	...	1.5
0.00625	0.09375	0.9	...	2.2
0.003125	0.096875	0.9	...	0.7
0.0	0.1	...	0.9	0.6
0.1	0.0	...	0.9	1.24
0.05	0.05	...	0.9	0.4
0.025	0.075	...	0.9	0.7

As in the case of diphtheria antitoxin, the antistreptococcus bodies are closely associated with the pseudoglobulins, as shown by the following experiment: 1,950 c.c. of antistreptococcus serum was precipitated with 2,385 c.c. of a saturated solution of ammonium sulphate, a proportion of 9:11 or a saturation of 55 per cent. The precipitate was pressed out, redissolved in water and the solution saturated with sodium chloride. This solution was filtered and the globulins precipitated by the addition of 2.75 c.c. acetic acid per liter. This precipitate was pressed out, dialyzed, neutralized, and filtered through a Berkefeld filter.[2] The material thus obtained was tested as to its opsonic activity when activated by fresh serum, and as to its protective power in guinea-pigs. When mixed with fresh serum in the proportion of nine to one, the point of opsonic extinction was reached at a dilution of 1:768 in the case of concentrated serum, as compared with 1:192 in the case of the original serum.

In guinea-pigs 0.5 c.c. of the concentrated serum protected against one M.L.D. of living culture, while one c.c. of the original serum was required to accomplish this. It remains to be determined whether the antistreptococcus bodies can be secured in greater concentration by employing the methods elaborated by Banzhof for the concentration of diphtheria antitoxin.

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[1] "Untersuchungen über Streptokokken u. Antistreptokokken-Serum," *Berl. klin. Wchnschr.*, 1902, 39, pp. 979, 1006.

[2] This concentration was made by P. G. Heinemann of the University of Chicago.

EFFECTS OF TRIKRESOL ON PHAGOCYTOSIS.

If we undertake the estimation of the opsonic content of serums containing trikresol by the usual method we may obtain erroneous results because of the inhibiting action of the preservative on the leukocytes. That the action of the preservative is on the leukocytes and not on opsonin is shown by the fact that a non-virulent streptococcus suspended in salt solution in the absence of serum is taken up by leukocytes to a considerable extent, while if 0.3 per cent trikresol is added phagocytosis ceases. Nine parts of a 0.3 per cent solution of trikresol in physiologic salt solution added to one part of active serum inhibits phagocytosis, while one part of the former solution added to nine parts of active serum does not. The exact concentration of trikresol required to check phagocytosis lies about midway between these two points.

The influence of trikresol in the estimation of opsonic power in the usual way is shown in the following results obtained with three fresh antistreptococcus serums with and without the addition of trikresol:

891—without trikresol = phagocytic index 4.8
893— " " = " " 2.6
1,130— " " = " " 3.0
891—with 0.4 per cent trikresol = phagocytic index 0.0
893— " 0.4 " " = " " 0.0
1,130— " 0.4 " " = " " 0.0

Hence in estimating the opsonic power of serums containing trikresol they must first be diluted to a point where the preservative becomes inactive. If the method of dilution to the point of opsonic extinction is used this source of error is eliminated.

In estimating the degree to which antistreptococcus serums may be reactivated by fresh serum the influence of preservatives must also be remembered. In the following experiment 0.4 per cent trikresol was added to an old antistreptococcus serum just before making the mixtures. The possible error in estimating the degree of reactivation by the usual method is apparent.

TABLE 4.

	Normal Fresh Serum	Leukocytic Suspension	Strepto-coccus Suspension	Average Bacteria per Phagocyte	Number of Cells in 50 Taking Part
891 without trikresol, 9 parts	1 part	1 part	1 part	4.6	23
893 " " 9 parts	1 part	1 part	1 part	1.2	10
1,130 " " 9 parts	1 part	1 part	1 part	4.3	23
891 with trikresol, 9 parts	1 part	1 part	1 part	0.06	2
893 " " 9 parts	1 part	1 part	1 part	0.06	2
1,130 " " 9 parts	1 part	1 part	1 part	0.0	0

PROTECTIVE ACTION IN GUINEA-PIGS.

The serums from the three horses were tested for three successive months, as soon as possible after being received, for their power to protect guinea-pigs against the virulent "Streptococcus S." The serum in varying doses was injected subcutaneously and on the following day one M.L.D. of the culture was injected intraperitoneally. This method was chosen to allow the removal and examination of the peritoneal exudate at intervals after the injection. The dose of the 24-hour broth culture required to kill all guinea-pigs from 250 to 350 gms. in weight varied from 0.02 to 0.03 c.c. In most tests 0.025 c.c was the amount injected.

The amount of serum required to protect against such a dose of streptococci varied among the horses and in the same horse at different months. In most instances seven c.c. of serum gave perfect protection, several times three c.c. were sufficient and in a few cases one c.c. was enough. No protection was secured with less than one c.c. of any serum. Even with an excess of serum the dose of culture could not be increased appreciably without breaking down the protection. The number of cocci contained in the M.L.D. seems to be a factor of importance, only a certain number being disposed of by the leukocytes which migrate into the peritoneal cavity. If the number of cocci injected is so large that they are able to multiply before there are enough leukocytes to take them up, the infection progresses and usually ends fatally. Guinea-pigs which have been perfectly protected against the injected streptococci have no free cocci in the peritoneal cavity after 24 hours. Often none can be found after four or five hours. In guinea-pigs which were partially protected the cocci were not entirely destroyed at once, and those remaining free multiplied and were only disposed of after two to four days. These animals behave like over-resistant normal ones, the injected serum placing them in a condition to overcome the infection but not affording complete protection.

The results obtained through examination of peritoneal exudates in injected animals have corresponded to those obtained and described by Bordet[1] in his classical paper on this subject.

In perfectly protected animals there was a prompt and abundant migration of leukocytes accompanied by rapid phagocytosis of the cocci. The cocci were soon destroyed in the cells, first staining poorly and then disappearing entirely. At the end of 24 hours no free cocci were seen, and often in four hours few could be seen even in the phagocytes. When the bacteria had been disposed of abundant endothelial cells appeared in the exudate and they in turn took up the leukocytes. In partially protected and very resistant normal animals the bacteria were taken up in part by the leukocytes which were less abundant than in the former case. The free bacteria multiply and the final result as to the fate of the animal depended on whether the bacteria or leukocytes finally obtained the upper hand. So long as there were many free cocci and much phagocytosis by leukocytes, there was no considerable increase of endothelial cells, which appear to perform their services after the struggle between the leukocytes and bacteria is over. In unprotected animals, and in most normal ones the bacteria multiplied rapidly, there were few leukocytes and little phagocytosis, and at the time of death the exudate contained few leukocytes and enormous numbers of free streptococci.

The character of the peritoneal exudate in individual animals is briefly shown in Tables 7 and 8.

The protective power of the serums in guinea-pigs corresponded with the ability of being reactivated by fresh serum. Serums which at first protected the animal against the living culture of streptococci lost this property when they were no longer capable of being reactivated. We have tested the activity of reactivated antistreptococcus serum upon different streptococci, and have observed nothing which would point toward any selective action on any of the different strains of streptococci used.

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[1] "Contribution à l'étude du sérum antistreptococcique," *Ann. de l'Inst. Pasteur*, 1897, 11, p. 177.

ALTERATIONS IN LEUKOCYTES AND OPSONIN IN INJECTED GUINEA-PIGS.

Guinea-pigs received subcutaneously six c.c. of an old antistreptococcus serum, being a dose slightly in excess of that required to protect against one M.L.D. of virulent streptococci injected into the peritoneal cavity 24 hours later. Such animals were examined at intervals as to the number and activity of the leukocytes in the peripheral blood, and as to alterations in the opsonic power of the serum for virulent streptococci. No increase in leukocytes was observed. There was an increased phagocytic activity of the leukocytes which disappeared 24 to 48 hours after the injection. This increased leukocytic activity appears to be specific for streptococci. The opsonic power of the serum of circulating blood for streptococci was increased, beginning one hour after injection of the serum and persisting for about 10 days. Table 5 shows the results obtained in such an experiment.

TABLE 5.

	Before Injection	Interval after Injection											
		1 Hour	2 Hours	3 Hours	4 Hours	16 Hours	2 Days	3 Day	4 Days	6 Days	8 Days	10 Days	16 Days
Opsonic index	0.5	0.9	1.3	1.2	1.3	2.1	2.3	2.3	3.2	2.4	1.5	1.6	0.4
Phagocytic activity of leukocytes as compared with normal...........	1.0	...	...	...	...	2.1	2.5	0.7	0.7	...	...	...	...

In animals injected with corresponding amounts of normal horse serum, no such alterations in opsonin and leukocytic activity were observed.

When the antistreptococcus serum is injected into the peritoneal cavity there follows a leukocytosis, associated with alterations in opsonic and leukocytic activity similar to those following subcutaneous injections.

DURATION OF PROTECTION.

With the object of learning the duration of the protection afforded by antistreptococcus serum, a series of guinea-pigs were each injected with six c.c. of an active serum subcutaneously.

After varying intervals they received one M.L.D. of culture intraperitoneally. The protection was found to persist for about eight days (Table 6).

This corresponds very closely with the period during which an elevation of opsonic power was demonstrable in the blood of animals which had received the serum.

TABLE 6.

POLYVALENT ANTISTREPTOCOCCUS SERUM FOLLOWED BY ONE M.L.D. OF LIVING STREPTOCOCCUS CULTURE.

Pig No.	Weight in Grams	Antistreptococcus Serum—1,130—Subcutaneously	"Streptococcus S," 24-Hour Broth Culture—Intraperitoneally	Interval between the Two Injections	Result
1	250	6 c.c.	0.025 c.c.	0	Died
2	255	"	"	1 hour	"
3	265	"	"	2 hours	"
4	270	"	"	3 "	"
5	300	"	"	4 "	Survived
6	250	"	"	17 "	"
7	280	"	"	1 day	"
8	270	"	"	2 days	"
9	250	"	"	3 "	"
10	260	"	"	4 "	"
11	260	"	"	5 "	"
12	250	"	"	6 "	"
13	265	"	"	"	"
14	270	"	"	10 "	Died
15	290	"	"	12 "	"

COMMERCIAL ANTISTREPTOCOCCUS SERUMS.

The methods of study which we have related as applied to the serums of individual horses were also used in the study of five commercial antistreptococcus serums; three of American and two of European manufacture. A résumé of the results secured with the American serums is shown in Table 7. Serum P (Parke, Davis & Co.) afforded complete protection against one M.L.D. of the culture in doses of two and six c.c., serum M (H. K. Mulford Co.) in doses of one, two, and six c.c., serum X had little protective power, the test animals corresponding quite closely to the control. Serum M did not protect in doses of less than one c.c. In this as in other tests normal horse serum appeared to lower the resistance, or it at least had no protective power.

Of the two antistreptococcus serums of European manufacture examined, one (Aronson's 20-fold) protected against one M.L.D. of the cultures in doses of one to six c.c., and the other (Institut Pas-

TABLE 7.

GUINEA-PIGS	WEIGHT IN GRAMS	COMMERCIAL ANTISTREPTOCOCCUS SERUMS—SUBCUTANEOUSLY FEB. 13, 1911 4 P.M.			NORMAL HORSE SERUM—SUBCUTANEOUSLY FEB. 13, 1911, 4 P.M.	"STREPTOCOCCUS S," 24-HR. BROTH CULTURE—INTRAPERITONEALLY FEB. 14, 1911, 5 P.M.	ANTISTREPTOCOCCUS SERUMS REACTIVATED WITH NORMAL FRESH HUMAN SERUM 1 PART IMMUNE TO NORMAL 9 PARTS OPSONIN			OPSONIN IN SERUM OF GUINEA-PIGS				FEBRUARY 15, 9:30 A.M.		FEBRUARY 16, 9 A.M.		FEBRUARY 17, 10 A.M.		FEBRUARY 18, 9 A.M.	FATE OF PIGS
										Before Serum Was Injected		24 Hrs. after Serum Was Injected									
		P	M	X			No. of Leukocytes out of 50 Taking Part	Average No. of Cocci per Phagocyte	Point of Opsonic Extinction	No. of Leukocytes out of 50 Taking Part	Average No. of Cocci per Phagocyte	No. of Leukocytes out of 50 Taking Part	Average No. of Cocci per Phagocyte	General Condition	Peritoneal Exudate	General Condition	Peritoneal Exudate	General Condition	Peritoneal Exudate		
1...	240	1 c.c.	.....	.....	.....	0.02 c.c.	21	4.2	1:384	1	0.04	8	1.8	Very sick	F.C., +++ L., ++ P., ++ E., 0	Dead	....	....	....	.....	Died
2...	260	2 c.c.	.....	.....	.....	0.02 c.c.	..	....	.....	3	0.1	11	1.5	Lively	F.C., 0 L., ++ P., 0 E., +	Lively	F.C., 0 L., +++ P., 0 E., ++	Lively	F.C., 0 L., + P., 0 E., +++	Lively	Survived
3...	280	6 c.c.	.....	.....	.....	0.02 c.c.	..	....	.....	4	0.2	10	0.8	Lively	F.C., 0 L., ++++ P., + E., ++	Lively	F.C., 0 L., +++ P., 0 E., ++	Lively	F.C., 0 L., + P., 0 E., +++	Lively	Survived
4...	240	.....	1 c.c.	.....	.....	0.02 c.c.	28	2.9	1:384	0	0.0	2	0.1	Lively	F.C., 0 L., ++++ P., 0 E., ++	Lively	F.C., 0 L., +++ P., 0 E., ++	Lively	F.C., 0 L., + P., 0 E., +++	Lively	Survived
5..	265	.....	2 c.c.	.....	.....	0.02 c.c.	....	....	.....	1	0.06	7	1.4	Lively	F.C., 0 L., ++++ P., 0 E., ++	Lively	F.C., 0 L., +++ P., 0 E., ++	Lively	F.C., 0 L., + P., 0 E., +++	Lively	Survived

6..	290	.....	6 c.c.	.....	.....	0.02 c.c.	....	....	.....	0	0.0	6	1.6	Lively	F.C., o L., +++ P., + E., +	Lively	F.C., o L., +++ P., + E., ++	Lively	F.C., o L., +++ P., o E., +++	Lively	Survived
7..	250	.....	.....	1 c.c.	.....	0.02 c.c.	7	0.98	1:48	1	0.06	1	0.08	Sick	F.C., ++ L., ++ P., + E., o	Fairly lively	F.C., + L., ++++ P., ++ E., +	Fairly lively	F.C., + L., ++ P., + E., ++	Lively	Survived
8..	270	.....	.....	2 c.c.	.....	0.02 c.c.	....	....	.....	2	0.16	2	0.1	Sick	F.C., ++ L., +++ P., + E., o	Fairly lively	F.C., + L., ++ P., ++ E., +	Fairly lively	F.C., o L., ++++ P., + E., ++	Lively	Survived
9..	315	.....	.....	6 c.c.	.....	0.02 c.c.	....	....	.....	0	0.0	5	0.8	Slightly sick	F.C., ++ L., +++ P., + E., o	Fairly lively	F.C., + L., +++ P., + E., +	Fairly lively	F.C., o L., +++ P., o E., ++	Lively	Survived
10..	300	.....	.....	.....	6 c.c.	0.02 c.c.	9	0.8	1:96	0	0.0	3	0.9	Very sick	F.C., +++ L., ++ P., ++ E., o	Dead	....	....	....	.....	Died
11..	320	.....	.....	.....	.....	0.02 c.c.	....	....	.....	0	0.0	0	0.0	Sick	F.C., ++ L., ++ P., ++ E., o	Fairly lively	F.C., + L., +++ P., + E., +	Fairly lively	F.C., + L., +++ P., + E., ++	Lively	Survived

Tests of commercial antistreptococcus serums: F.C. = free cocci; L. = leukocytes; P. = phagocytosis; E. = endothelium.

teur) in doses of two to six c.c. Smaller doses were without effect. It is thus apparent that the two active American serums possessed a protective power equal to that of the European serums.

Our former failures to secure any protection with commercial antistreptococcus serums, it is now believed, may have been due to lack of appreciation of the importance of the relation between the degree of immunity and the number of bacteria injected.

It is not unlikely that the number of bacteria contained in a M.L.D. of the culture is an important factor in determining the amount of serum required for protection. We have estimated by dilution and plate cultures that one M.L.D. of our culture contains about 12,500,000 cocci. Of the culture used by Bordet in his test of the action of antistreptococcus serums in rabbits, the M.L.D. intraperitoneally was 0.000,001 c.c. or approximately 500 cocci. The culture employed by Aronson in standardizing his antistreptococcus serum in mice possesses a M.L.D. of 0.000,000,05 c.c. or approximately five cocci. Since protection is apparently dependent on rapid and complete destruction of the cocci after injection, it appears reasonable that with an equal quantity of available immune bodies, opsonification and subsequent phagocytic destruction would be more likely if very few cocci are present. For this reason the most highly virulent cultures would seem to be the best suited for measuring comparatively low degrees of immunity. With such cultures in susceptible animals, 10 or 100 times the M.L.D. contains relatively few cocci.

It is of interest to note that the serums which were reactivated to a considerable degree were also the ones which afforded protection to the animals. In the animals which showed complete protection the opsonic power in the blood rose after the injection of the serum, while in the others this did not occur except in one case (guinea-pig 1).

OLD COMMERCIAL SERUM.

Five commercial antistreptococcus serums which had been in the ice-box for over two years and whose dates for use had expired were tested in a similar manner. The results are given in Table 8.

TABLE 8.

GUINEA-PIGS	WEIGHT IN GRAMS	OLD ANTISTREPTOCOCCUS SERUM—SUBCUTANEOUSLY FEB. 27, 1911, 5 P.M.					OLD NORMAL HORSE SERUM—SUBCUTANEOUSLY FEB. 27, 1911, 5:00 P.M.	"STREPTOCOCCUS S." 24-HR. BROTH CULTURE—INTRAPERITONEALLY FEB. 28, 1911, 3:30 P.M.	POINT OF OPSONIC EXTINCTION OF SERUMS REACTIVATED WITH NORMAL HUMAN SERUM; 1 IMMUNE TO 9 NORMAL	OPSONIC INDEX OF SERUM OF GUINEA-PIGS		MARCH 1, 9 A.M.		MARCH 2, 9 A.M.		FATE OF PIGS
		A	H	M	S	C				Before Serum Was Injected	18 Hrs. after Serum Was Injected	General Condition	Peritoneal Exudate	General Condition	Peritoneal Exudate	
1.....	250	6 c.c.	.....	.....	.....	.....	.....	0.025 c.c.	1:1536	.3	1.4	Lively	F.C., 0 L., ++++ P., 0 E., +++	Lively	F.C., 0 L., + P., 0 E., +++	Survived
2.....	250	.....	6 c.c.	.....	.....	.....	.....	0.025 c.c.	1:48	1.0	0.9	Very sick	F.C., ++++ L., ++ P., +++ E.,	Dead	....	Died
3.....	250	.....	.....	6 c.c.	.....	.....	.....	0.025 c.c.	1:384	.3	1.7	Lively	F.C., 0 L., ++++ P., + E., ++	Lively	F.C., 0 L., + P., 0 E., +++	Survived
4.....	250	.....	.....	.....	6 c.c.	.....	.....	0.025 c.c.	1:768	.3	1.6	Lively	F.C., 0 L., ++++ P., 0 E., +++	Lively	F.C., 0 L., ++ P., 0 E., +++	Survived
5.....	260	.....	.....	.....	.....	6 c.c.	.....	0.025 c.c.	1:48	.3	1.5	Quite sick	F.C., ++ L., ++ P., + E.	Very sick	F.C., ++ L., ++ P., + E.	Died
6.....	220	.....	.....	.....	.....	.....	6 c.c.	0.025 c.c.	.....	1.0	1.0	Very sick	F.C., +++ L., +++ P., +++ E.	Dead	....	Died
7.....	255	.....	.....	.....	.....	.....	.....	0.025 c.c.	.....	...	...	Quite sick	F.C., ++ L., +++ P., ++ E.	Quite sick	F.C., + L., ++ P., +++ E.	Survived

Tests of antistreptococcus serums over two year old: F.C.=free cocci; L.=leukocytes; P.=phogocytosis; E=endothelium.

CURATIVE POWER IN GUINEA-PIGS.

We have been unable to observe any curative effects from serums given after the injection of the cultures, and if the serums were given less than four hours before the culture no protection was manifest.

The rapidity with which a streptococcus infection in guinea-pigs runs its course and terminates in death or recovery makes it difficult to study in this animal the curative power of antistreptococcus serum. The curative effects described in animals as related by several workers have been secured only if the serum was given very soon after the infection had been produced and not later than a few hours.

PROTECTIVE AND CURATIVE ACTION ON MAN.

We have pointed out that the antistreptococcus serums are reactivated more readily and to a greater degree by fresh human that by fresh guinea-pig serum. This may explain the relatively large dose of serum required to protect the guinea-pig and may indicate a relatively greater protective and curative power in man from the same serum.

The ability of fresh blood serum to reactivate an antistreptococcus serum appears to furnish quite an accurate index to the probably protective activity of the serum when injected in the guinea-pig. It may be inferred that this will also hold good in man and that individuals may be protected from subsequent streptococcus infections by the injections of suitable quantities of antistreptoccus serum.

Determination of the opsonic content of the blood of the injected individual may serve to indicate when enough serum has been given to produce protection or to have a curative effect, as in guinea-pigs protected in this way the opsonin in the blood serum was appreciably increased. Since reactivation of antistreptococcus serum by fresh human serum is not obtained at a very high dilution, it probably follows that the efficient dose in man must be rather large. This is in accord with the experience of those who have secured favorable results in the clinical use of the serum.

It is very difficult to compare the therapeutic effects of antistreptococcus serum in animals with their rapidly fatal generalized infections with those in man with the usually more prolonged and localized disease.

In cases of streptococcus infection in man we have several times found that the fresh serum of the individual is still able to reactivate antistreptococcus horse serum, even though the patient's blood contains much less opsonin for streptococci than normal. This would suggest that the immune serum would be active against the infection.

In normal guinea-pigs the injection of antistreptococcus serum has a direct stimulating effect on the phagocytic activity of the leukocytes. In some cases of streptococcus infection in man the leukocytes are reduced in their phagocytic power, and are relatively inactive in the presence of opsonin. It would be of interest to know whether the antistreptococcus serum may be useful in restoring the activity of the leukocytes in such cases.

STANDARDIZATION OF ANTISTREPTOCOCCUS SERUM NEEDED.

In view of the fact that one of the three antistreptococcus serums which were tested was almost entirely lacking in any immune bodies as indicated by the animal tests and also by the reactivation experiments, it would seem desirable to have some guarantee that such serums when offered for sale are active. If possible a standard should be established by the Hygienic Laboratory, United States Public Health and Marine Hospital Service. We believe that estimations of the reactivability of immune serums by fresh serum using a virulent culture in making the determinations would be a distinct aid in such standardization. Whether this method could be also applied to such serums as that of Moser, in the preparation of which cultures non-virulent for animals are employed, we have not had an opportunity of learning.

CONCLUSIONS.

Antistreptococcus serums rapidly lose their opsonic power, which may for some time be largely restored by the addition of fresh human or guinea-pig serum.

Guinea-pigs may be protected against virulent cultures of streptococci by previous injection of antistreptococcus serums.

The protective power of immune serums continues so long as they can be reactivated by fresh serum.

Injections of immune serums in guinea-pigs may be followed by increased activity of leukocytes of short duration and by an increased opsonic power for streptococci in the blood serum persisting for about 10 days.

The immunity in guinea-pigs produced by injection of immune serum persists for about eight days.

Of three fresh commercial serums manufactured in the United States which were tested two were active and one inactive. The two European serums were active.

Fresh normal human serum and fresh human serum from persons infected with streptococci are able to reactivate antistreptococcus serums. This indicates that such serums may have some protective and curative effect in man in cases of streptococcus infections.

The specific antistreptococcus bodies are resistant to heat and chemicals (trikresol, 0.4 per cent chloroform) and are closely associated with the pseudoglobulins of the immune serum.

It would be desirable to have some guarantee of the activity of antistreptococcus serums offered for sale.